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Insecticide Resistance Study in *Plutella xylostella* (L.)

V. The Induction, Cross Resistance and Glutathione-S-transferase in Relation to Mevinphos-resistance[1]

by

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Abstract : The diamondback moth, *Plutella xylostella* (L.), developed resistance to mevinphos rather slow and unstable. After twenty generations of mevinphos suppression at LC_{75} level, only 5-8 folds resistance was obtained. The mevinphos-resistant diamondback moth has a broad cross resistance to other organophosphorus compounds and cartap, but this resistance was not extended to most of the synthetic pyrethroids. Significant increase of glutathione-S-transferase activity occurred in the 4th instar and was paralleled with a reduction in mevinphos susceptibility during the same period. The age related glutathione-S-transferase activity change was also observed in the mevinphos-resistant strain except the overall enzyme activity was at higher level.

Introduction

Mevinphos is officially recommended for controlling the diamondback moth, *Plutella xylostella* (L.), in Taiwan. Several major vegetable cultivation areas have adapted this insecticide for many years due to its effectiveness and short residue period ; undoubtfully, mevinphos has also confronted with resistance in the diamondback moth as any other synthetic insecticide does.

In a general survey performed in 1980-81 [4], we found that the mevinphos-resistance in the wild diamondback moth populations can still be considered mild in contrast with the high resistances to carbofuran and synthetic pyrethroids, despite they were introduced for field use much later. The resistance response of the diamondback moth to mevinphos is a very important matter but was never carefully studied before. Following the 1980-81 survey, we started to evaluate the induction rate and the cross resistance characters of mevinphos-resistance. The information will help us in understanding the consequences in regard to mevinphos application for the diamondback moth control

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and improving the undesirable aspects. Recently, we found the activity of glutathione(GSH)-S-transferase, a detoxication enzyme for organophosphorus, was increased in both the mevinphos- and profenofos-resistant strain diamondback moths[6] and the enzyme was investigated thoroughly in this study for its role and implication in relation to the organophosphorus resistance.

Materials and Methods

The IL-strain diamondback moth of our 1980-81 collections was used as the susceptible strain due to its high sensitivity to mevinphos. The IL-strain was also used as the parental strain for mevinphos resistance induction. Usually, one thousand late 3rd instar larvae were sprayed with mevinphos and the survived insects were collected for propagating the next generation. The press pressure was set at LC_{75} which was re-determined in each generation on 100 to 200 early emerged larvae of that generation. The increase of LC_{75} from generation to generation were counted as the resistance induction rate.

Fourteen commercially formulated insecticides listed in Table 1 were used for the cross resistance test. The cross resistance of mevinphos-resistant strain was studied in the same way as previously reported[7]. Insects were carefully selected and separated into four age groups, i.e., the 3rd, the early 4th, the late 4th instar larvae and the early pupal stage for GSH-S-transferase study. The enzymes were prepared and assayed as before [1,6] and the proteins were determined by the Lowry method[9].

Results

After twenty generations of mevinphos suppression at LC_{75} level, only 5-8 folds resistance was obtained in the diamondback moth. The resistance development was presented in Fig. 1 with suppression concentrations indicated in Y-axis and the diamondback moth generations in X-axis. The actual mortality following each press was noted in

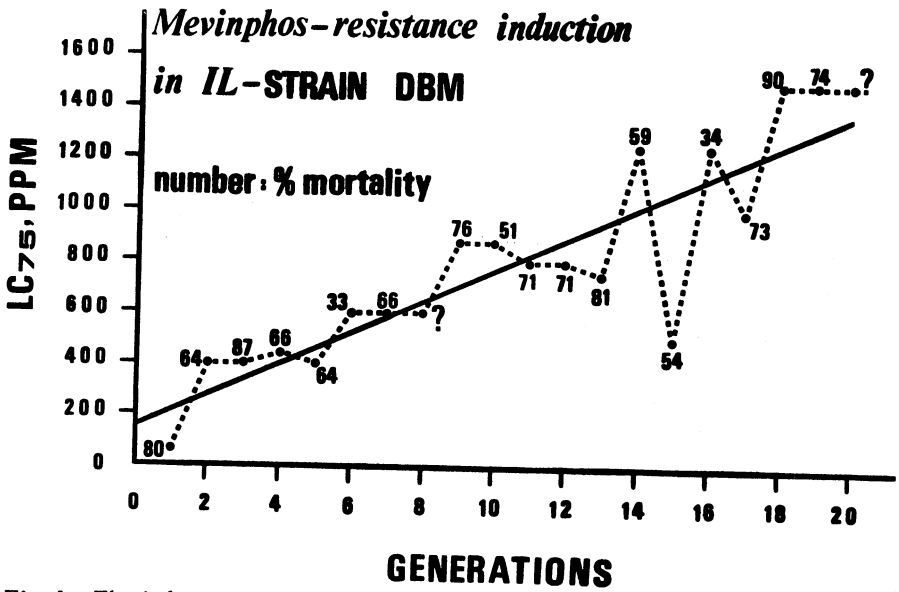

Fig. 1. The induction of insecticide resistance in the diamondback moth by mevinphos on each treatment at the selection pressure of LC_{75} with actual mortality indicated

the graph. Diamondback moth developed resistance to mevinphos much slower than that of carbofuran, cartap and fenvalerate[7]. The mevinphos-resistant strain has obtained a level of resistance equivalent to that of the most resistant LC-strain[4] and is ready for the following tests.

The cross resistance of mevinphos-resistant strain to fourteen commonly used insecticides was listed in Table 1.

Table 1. The cross resistance of mevinphos-resistant diamondback moth to fourteen insecticides

Insecticides	S-strain		R-strain		R/S[a]	
	LC_{50}	LC_{75}	LC_{50}	LC_{75}	LC_{50}	LC_{75}
Organophosphorus						
Cyanofenphos	190	400	686	1,647	3.6	3.7
Diethquinalphion	230	500	1,289	2,800	5.6	5.8
Mephosfolan	360	900	608	1,521	1.7	1.7
Methamidophos	560	1,400	713	1,269	1.3	0.9
Phenthoate	670	1,700	589	1,793	0.9	1.1
Profenofos	100	230	444	1,040	4.4	4.5
Prophos	170	260	826	2,720	4.9	10.5
Prothiophos	310	560	643	1,475	2.1	2.6
Synthetic pyrethroids						
Cypermethrin	19.0	53.0	30.9	107.5	1.6	2.0
Decamethrin	3.6	11.0	12.4	44.2	3.5	4.0
Fenvalerate	8.3	23.0	15.2	31.2	1.8	1.4
Permethrin	14.0	30.0	38.4	65.4	2.7	2.2
UCX-33[b]	63.0	160.0	108.1	400.5	1.7	2.5
Others						
Cartap	290	580	1,804	4,203	6.2	7.3

a R/S for mevinphos-resistance is 5.6 for LC_{50} and 8.3 for LC_{75}

b Permethrin, formulated by Union Carbide Co.

In general, organophosphorus compounds were the most affected group by the cross resistance from mevinphos, and the effectiveness lowered significantly in four out of eight tested organophosphorus insecticides. Prophos was affected mostly and followed by diethquinalphion, profenofos and cyanofenphos; prothiophos, phenthoate, mephosfolan and methamidophos were not affected. Except decamethrin, the synthetic pyrethroids were not affected significantly. Unexpectedly, cartap was seriously affected by mevinphos cross resistance and its implication on cartap-resistance in field diamondback

moth populations needs to be evaluated further.

We have observed that the GSH-S-transferase activity is higher in the organophosphorus-resistant diamondback moth than in the susceptible IL-strain[6]. A more detailed study was carried out in both the mevinphos-resistant and the susceptible IL-strain diamondback moths and the results were presented in Fig. 2. The GSH-S-transferase may play a very important physiological role in the growth of the diamondback moth because the activity increased rapidly during the 4th instar and the activity remained high in early pupal stage. From the 3rd instar to the early pupal stage, the GSH-S-transferase activity increased ten times in the susceptible IL-strain,

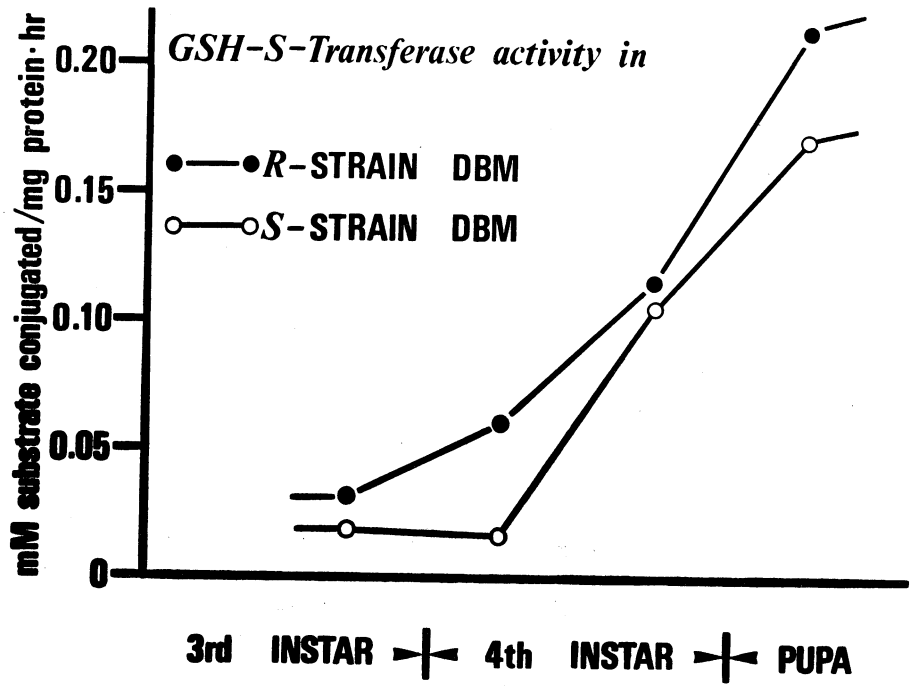

Fig. 2. Naturally occurred glutathione-S-transferase activity increases in the 4th instar larvae of both susceptible and mevinphos-resistant diamondback moths

and the same phenomenon was also observed in the mevinphos-resistant strain except at higher activity level (Fig. 2). We suspect this dramatic change of GSH-S-transferase may also altered the diamondback moth susceptibility to organophosphorus insecticides. In a subsequent test, organophosphorus susceptibilities at different larval instar were compared in the IL-strain diamondback moth, the results were presented in Table 2 along with the larval weight and body surface changes.

Table 2. The comparison of size, mevinphos susceptibility and GSH-S-transferase activity between the 3rd and the 4th instar diamondback moths

Instars	Weight (mg)	BodySurface (cm^2)	Weight/ BodySurface	LC_{50} (ppm)	LC_{50}/Weight/ Body Surface	Enz. Act.[a]
4th	5.8	0.160	36.3	632	17.4	0.106
3rd	1.6	0.072	22.2	108	4.9	0.019
4th/3rd	3.6	2.240	1.63	5.85	3.6	5.580

a. Enz. Act. : Glutathione-S-transferase activity in mM substrate conjugated/mg protein • hr

The larval weight increased 3.6 times from 3rd to 4th instar accompany with a body surface increase at 2.24 times. The morphological change increase the needed dosage by a factor of 1.63, but the actual increase is almost 6 folds. The discrepancy indicated that even the susceptible diamondback moth can become more resistant to organophosphorus insecticides by the age related physiological changes or as we have suspected, by the higher GSH-S-transferase detoxication rate.

Discussion

Chang has reported that field diamondback moth population from two intensively cultivated areas had high resistance to mevinphos and malathion[3]. Lee and Lee studied the organophosphorus resistance in the diamondback moth and found the diamondback moth collected from I-lan area was the most susceptible strain among ten areas they had sampled[8], which agreed with our 1980-81 survey[4]. They had found that the susceptibility was increased by rearing the diamondback moth indoors for several generations, but this phenomenon was not observed by us. They also induced a mevinphos-resistant strain having a resistant ratio of 10, which is similar to our result except that the press was performed on the adults rather than larvae. They observed high cross resistances in the malation-resistant strain to four other organophosphorus insecticides which agreed with our observation on mevinphos-resistant strain.

The slow and unstable development of mevinphos resistance in the diamondback moth may be a reason that made mevinphos still effective today in the diamondback moth control in many areas. The overall results of this study indicates that the diamondback moth can develop strong resistance to mevinphos and will result in serious cross resistance in organophosphorus compounds. The fact of both malathion and mevinphos can induce broad organophosphorus cross resistance may indicate that the diamondback moth have ability to generate a resistance which can respond to most organophosphorus insecticides.

After briefly comparing the structures of nine tested organophosphorus compounds, we found no sign of specific structure or functional group is responsible for the cross resistance. Instead, it seems that the whole organophosphorus group has lost its insecticidal action. The synthetic pyrethroids were not significantly influenced by the mevinphos cross resistance, only a three- to four-time increase in LC_{75} concentration was observed in decamethrin. We felt that the high synthetic pyrethroid resistance observed in fields was not related to the cross resistance from mevinphos.

Chang and Sun have concluded that the GSH-S-transferase was not involved in the detoxication of diazinon in the diamondback moth *in vitro* based on a soluble fraction

prepared more than an hour[2]; we have found the same enzyme was deteriorated quickly after a long preparation period[6]. Wu and Sun also have found fivetime difference in GSH-S-transferase activity between the wild and susceptible strain, they decided not to correlate that to the observed organophosphorus resistance because no clear insecticide application history can be followed for the wild strain[10].

After noticed the increase in GSH-S-transferase activity in mevinphos-pressed diamondback moth, we felt that this enzyme should be studied carefully. We have found the diamondback moth having a dramatic GSH-S-transferase activity change in its late larval stage, which parallel with a decrease in organophosphorus susceptibility during the same period. This unexpected GSH-S-transferase activity increase is a very interesting matter in regard to our investigation of diamondback moth detoxication ability. We suspected the inherited physiological increase of GSH-S-transferase may have connections with the difficulty in inducing mevinphos resistance during the late larval stage. Another fact that we cannot overlook is that the diamondback moth can transform itself from a susceptible to a resistant individual simply by the age change. After dividing the 6 folds dosage increase by the morphological factor change of 1.62, the susceptibility was reduced by a factor of 3.6 from 3rd to late 4th instar. During the same period, the GSH-S-transferase activity has increased 5 folds. We have no intention to interpret these two matters on their magnitudes change, but the possible correlation between them is worthy to be investigated.

Conclusion

Obviously, the slow and unstable induction of resistance in the diamondback moth by mevinphos has its advantage in pest management that we can justify the continuing use of this insecticide is still proper. Particularly, mevinphos degrades very fast on vegetable[5], which ensures the residue safety on marketed vegetables. Besides, the use of mevinphos will not interfere with the effectiveness of synthetic pyrethroids, which undoubtfully will be the major insecticides for vegetable usage in the future. However, we felt that the mevinphos resistance in the diamondback moth should be monitored closely in vegetable production areas because the cross resistance will jeopardize the effectiveness of other organophosphorus for diamondback moth control.

Naturally occurred GSH-S-transferase activity change in the diamondback moth larvae has made the usage of organophosphorus as control agent a very delicate matter. This study has revealed that the diamondback moth grown from the 3rd to late 4th instar was accompanied with a decline in organophosphorus susceptibility. Such a matter, to transform a susceptible population into a naturally immuned population, implied that the usage of organophosphorus insecticides in the diamondback moth control needs to be reevaluated. Particularly, the population composition of diamondback moth is usually a mixture in age groups. If the effectiveness of applied insecticide varied with the composition of age groups, the inconsistant effectiveness will affect the farmer psychologically and hence reduce their confidence on the officially recommended pest management methods.

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小菜蛾抗藥性之研究

V、抗美文松品系誘發之交互抗性及其 Glutatione-S-transferase 活性變化情形

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摘 要

採自宜蘭地區之感性品系小菜蛾對一般常用殺蟲劑感度均高，使用此品系測定對美文松抗葯性時，發現抗藥性之發展緩慢且不甚穩定，經過20世代，淘汰 75 %總蟲數之選擇，僅獲得 5 — 8 倍抗藥能力，較以往使用加保扶、芬化利及培丹誘發抗藥力爲緩慢。所得抗美文松品系小菜蛾對其他有機磷劑均能產生交互抗性，其中以普伏松之交互抗性最爲嚴重，其次爲拜裕松、佈飛松；美福松、達馬松及賽達松雖無顯著之交互抗性生成，然其原來之感度甚低，造成 9 種有機磷劑對抗美文松品系無良好殺蟲效果；對培丹亦有交互抗性，但對合成除蟲菊精類則無明顯之交互抗性發生。Glutathione-S-transferase 係一對有機磷類殺蟲劑之解毒酵素，其活性在抗藥品系中較高。研究中發現，該酵素在小菜蛾幼蟲期第四齡時有大幅度之變化，酵素活性由三到四齡增加 5 — 6 倍，四齡末以後至蛹期之酵素活性變化甚小，因此本試驗亦比較同爲感性品系之三、四齡幼蟲對美文松之敏感度，結果顯示該兩齡期幼蟲之敏感度隨 glutathione-S-transferase 活性的增加而降低，此一現象可能與解毒作用之自然增加有密切之關係，且對田間防治小菜蛾用藥劑量及其所能殺死之不同齡期小菜蛾幼蟲影響很大，因此在防治率之測定上，有值得再行檢討之必要。

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